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# COMPARATIVE HISTOLOGY OF ALFALFA AND CLOVERS

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(WITH EIGHT FIGURES)

Perennial leguminous forage plants are growing in importance both for green feeding and for hay, and some of them, notably alfalfa, red and alsike clovers, are well adapted for grinding into meal. The work detailed in this paper was undertaken to facilitate the microscopic identification of the species named in mixed cattle foods.

The highest feeding value of the hay or "meal" is obtained from plants cut in early flower, though the more or less mature fruits and seeds are not infrequently found in the products on the market, especially in alfalfa meal.

## Alfalfa

Alfalfa (*Medica sativa* [L.] Mill., *Medicago sativa* L.) is a native of Asia and has been cultivated for fodder since long before the Christian era. It is now grown in both hemispheres, especially in the arid and semiarid regions of the Southwest, for use either fresh or dried. As the hay is brittle, resulting, when fed from the bale, in a considerable loss of leaves, the product is often kiln-dried and ground to a meal.

Ordinary alfalfa, or lucerne, branches profusely and bears alternate leaves (fig. 1, I) consisting of three distinct obovate to lanceolate leaflets finely dentate at the apex. The plant is described as glabrous; hairs, however, are evident under a lens and are highly characteristic with higher magnifications. The flowers (fig. 1, II) appear in racemes of 8–25 each and wither after flowering. They are of the distinctly papilionaceous type, small (8–10 mm. in length),



FIG. 1.—Alfalfa (*Medica sativa*): I, leaf,  $\times 1$ ; II, flower,  $\times 3$ ; III, seed,  $\times 3$ ; IV, fruit,  $\times 3$ .

and delicate in structure. The hairy calyx consists of a tube and 5 teeth-like lobes of about the same length as the tube, at the base of which is inserted the violet-colored corolla. The 10 stamens are combined in two sets; the ovary is one-celled with several ovules. At maturity the brown pods (fig. 1, IV) are coiled 2-4 times in close spirals, the diameter of the coil being about 4 mm. The greenish-brown seeds (fig. 1, III), up to 3 mm. in length, are somewhat kidney-shaped.

Many varieties of alfalfa, less widely grown, vary in flower color, through blue, white, and green, to yellow, and in number of pod coils, seeds, and leaflets.

### HISTOLOGY

STEM (fig. 2).—The *epidermal cells* (*ep*) are several times longer than broad and arranged end to end in longitudinal rows interrupted frequently by stomata and their accompanying cells. The outer and inner walls are slightly thickened, the former having a cuticle with delicate striations evident in cleared preparations.

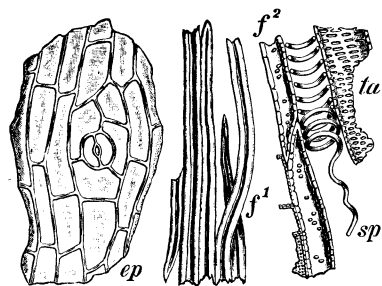


FIG. 2.—Alfalfa: elements of stem in surface view; *ep*, epidermis; *f*<sup>1</sup>, bast fibers; *f*<sup>2</sup>, wood fibers; *sp*, spiral vessels; *ta*, pitted vessels;  $\times 160$ .

*Bast*.—Several layers of simple thin-walled chlorophyll-bearing parenchyma cells, interrupted occasionally, especially at the angles of the stem, by masses of collenchyma, form the

outer tissues. Underlying this is a single layer of thin-walled crystal-bearing cells inclosing a zone of bast fiber bundles, each bundle being wedge-shaped in cross section. The individual fibers (*f*<sup>1</sup>) are greatly elongated and have walls so strongly thickened that the lumen is often but a mere line.

*Phloem*.—This consists of a characterless mass of thin-walled cambium cells and sieve tubes.

*Xylem*.—The most evident elements of this woody tissue are the pitted (*ta*) and spiral (*sp*) vessels and the pitted wood fibers (*f*<sup>2</sup>).

*Pith*.—This consists of comparatively large, thin-walled, pitted cells with no cell contents.

*LEAF*.—*Upper epidermis*.—The cells are approximately  $35\mu$  in diameter, although often elongated, especially over the veins. The cell walls are strikingly sinuous and of uniform thickness. Numerous simple stomata are scattered over the whole surface, and occasional hairs, similar to those so abundant on the lower surface, occur at the base of the leaf. Cuticular striations are very distinct in cleared material.

The *palisade layer* consists of very simple cells with breadth half the height.

*Mesophyll*.—The ground tissue of this layer is made up of a loose mass of parenchyma with no characteristic features; accompanying the simple bundles, however, are crystal-bearing cells (fig. 3, *cr*) of diagnostic importance. The latter are thin-walled and arranged more or less end to end in longitudinal rows. Each cell contains a single monoclinic crystal about  $18\mu$  in length, the facets of which often appear corroded.

The *lower epidermis* (fig. 3) differs from the upper principally in the greater number of hairs which are scattered over the whole surface and margin of the leaf, being especially numerous along the veins. The cells surrounding the hair base form a rosette. The hairs are of two kinds, unicellular (numerous) and capitate (scattered). The unicellular hairs (*t*<sup>1</sup>) are more or less sinuous, thick-walled, the lumen being a mere line, with small but prominent warts distributed over the entire length. They arise from a small, slightly thick-walled basal cell and average  $800\mu$  in length and  $15\mu$  in breadth, though the length varies up to over 1.5 mm. The

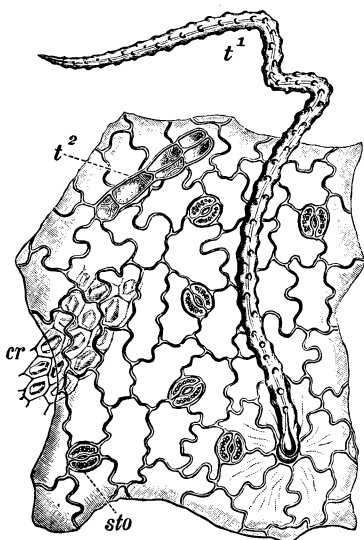


FIG. 3.—Alfalfa: lower epidermis of leaf with unicellular hair (*t*<sup>1</sup>), capitate hair (*t*<sup>2</sup>), and stoma (*sto*); *cr*, crystal cells accompanying bundles;  $\times 160$ .

capitate hairs ( $t^2$ ) consist of a stalk of two or three cells and a multicellular head, all the cells being thin-walled and frequently collapsed.

**CALYX.**—The *epidermis* bears unicellular and capitate hairs of the same general structure as those on the leaf. On the calyx tube the unicellular hairs are comparatively short and thick-walled, while on the lobes they are longer and thinner-walled, with correspondingly broader lumen. The simple bundle running out to the tip of each lobe is surrounded by a layer of crystal cells each containing a crystal averaging  $18\ \mu$  in length.

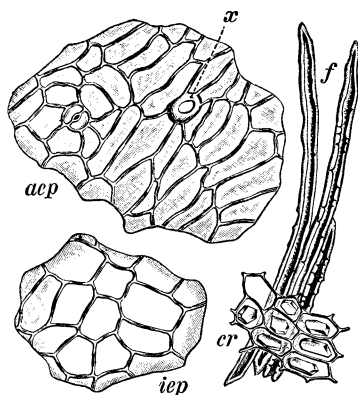


FIG. 4.—Alfalfa: elements of pod in surface view; *aep*, outer epidermis with hair scar ( $x$ ); *iep*, inner epidermis; *cr*, crystal layer; *f*, fibers;  $\times 160$ .

**COROLLA.**—The *epidermal cells* of the petals, at the base, are very thin-walled, elongated, and somewhat sinuous, and bear toward the tip papillae with striated cuticle. The bundles are very small, often but a single spiral vessel marking their course.

**STAMENS.**—The *filaments* consist of delicate cells similar to those of the petal in structure. The *anthers* have riblike thickenings over their whole surface.

**PISTIL.**—The *stigma* bears colorless papillae closely matted together.

The *style* is made up of small characterless cells except the outer half, which is covered with cells slightly thickened, apparently for mechanical support.

**Ovary.**—The small thin-walled epidermal cells bear numerous thin-walled unicellular and capitate hairs.

**PERICARP** (fig. 4).—The *epicarp* (*aep*) consists of a single layer of empty cells usually more or less elongated except at the stomata, about which they form a rosette. Hairs are frequently present, but often break off from the dried pod, leaving a scar ( $x$ ) with a thickened wall.

**Mesocarp.**—The characteristic tissues are: crystal cells (*cr*)

with very thin walls, frequently side by side in rows, each cell containing a single crystal, and fibers (*f*) with rather blunt ends and pitted walls, the number of pits being most numerous in fibers with the thickest walls.

*Endocarp (iep)*.—A single layer of epidermal cells without stomata completes the pericarp.

**SPERMODERM** (fig. 5, *S*; fig. 6).—The *palisade cells (pal)* are upward of  $35\ \mu$  high and  $8\text{--}10\ \mu$  broad, with rounded outer ends and a thin cuticle. A narrow light line (*l*), situated about  $7\ \mu$  within the outer end, can be easily seen in cross section. As is

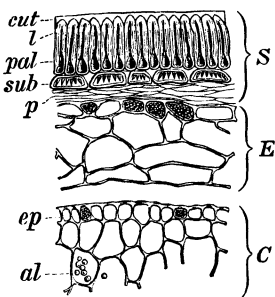


FIG. 5.—Alfalfa: seed in cross section; *S*, spermoderm consists of palisade cells (*pal*) with cuticle (*cut*) and light line (*l*), subepidermal layer (hour-glass cells) (*sub*), and parenchyma (*p*); *E*, endosperm; *C*, cotyledon with epidermis (*ep*) and aleurone grains (*al*);  $\times 160$ .

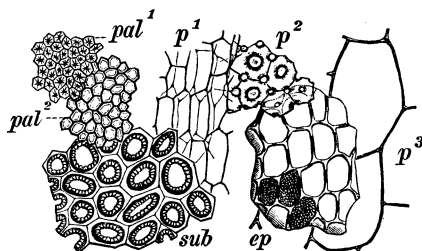


FIG. 6.—Alfalfa: elements of seed in surface view; *pal*<sup>1</sup>, outer palisade cells; *pal*<sup>2</sup>, inner palisade cells; *sub*, subepidermal layer (hour-glass cells), and *p*<sup>1</sup>, *p*<sup>2</sup>, parenchyma of spermoderm; *ep*, epidermis, and *p*<sup>3</sup>, parenchyma of endosperm;  $\times 160$ .

usual in legumes, the outer cell walls are greatly thickened, showing a radiating cavity (*pal*<sup>1</sup>) in surface view, while the inner portion of the cells has thinner walls and correspondingly broader lumen (*pal*<sup>2</sup>).

*Subepidermal cells (sub)*.—These cells, although only about  $6\ \mu$  high over the greater part of the seed, are very broad (upward of  $30\ \mu$ ) and are especially striking because of their prominent ribs clearly evident in surface view, where they present a beautiful radiating effect. In cross section they show evidence of the hour-glass form so characteristic of many legumes, the inner ends being broader than the outer.

The *parenchyma* (*p*) consists of several layers of compressed cells. The outer layers are of simple thin-walled parenchyma without intercellular spaces (*p*<sup>1</sup>), while the inner layers are often distinctly spongy with evident intercellular spaces (*p*<sup>2</sup>).

ENDOSPERM (fig. 5, *E*; fig. 6).—A simple epidermal layer (*ep*) containing aleurone grains is followed by several layers of large, more or less collapsed, empty cells with thin walls (*p*<sup>3</sup>).

EMBRYO (fig. 5, *C*).—The cotyledons have a small-celled epidermis and mesophyll containing aleurone grains (*al*) but no starch. Palisade cells underlie the inner epidermis.

### Red clover

Red clover (*Trifolium pratense* L.) is indigenous to Europe and is extensively grown for fodder in the United States, where it also grows spontaneously, having escaped from cultivation.

The pubescent stems are ascending, with 3-foliate toothed leaves, each oval leaflet often being notched at the apex and marked on the upper surface with a whitish spot. The rose-red flowers are borne in a dense sessile head closely surrounded by the uppermost leaves. The persistent calyx, with 5 bristle-like teeth and a bearded ring in the throat, is nearly as long as the delicate papilionaceous corolla, which is tubular below and withers after flowering. The pod differs from alfalfa in that it is one-seeded, straight, and flattened oval, with a very thin membranous lower half and hard caplike top. The seeds are slightly smaller than those of alfalfa, averaging 2 mm. in length. They are flattened kidney-shaped or rounded triangular with unequal sides one of which is concave. The color varies through light yellow to purple, the individual seeds being uniform in color or variegated.

### HISTOLOGY

STEM.—The *epidermal cells* are longitudinally elongated with straight walls, often beaded especially just below the nodes, and a striated cuticle. Interspersed among these cells are numerous stomata and both unicellular and capitate hairs. The unicellular hairs, like those on the leaf, are long, thick-walled, warty, and

borne on a characteristic swelling of the epidermis having the appearance of an emergence.

*Bast*.—The only noteworthy tissues are the crystal-bearing cells accompanying the bundles of bast fibers and the large air spaces, below the unicellular hairs, such as occur on the leaf.

*Phloem*, *xylem*, and *pith* are similar to those of alfalfa.

*LEAF*.—The *upper epidermis* consists of approximately isodiametric cells with thin, gently wavy walls and scattered stomata. Hairs are absent.

*Mesophyll*.—The small bundles running through the simple parenchymatous ground tissue are accompanied by crystal-bearing cells, each cell containing a monoclinic crystal averaging  $16\ \mu$  in length.

The *lower epidermal cells* (fig. 7) have sinuous walls, the rather sharp bend of the waves being thickened and sometimes extended into the cell cavity as projections. Highly characteristic are the hornlike projections about the stomata (*sto*). The walls become slightly thicker and usually pitted toward the base of the leaf, especially

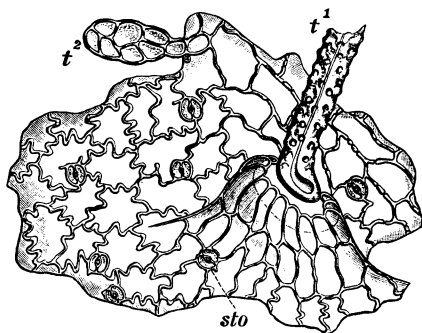


FIG. 7.—Clover (*Trifolium pratense*): lower epidermis of leaf with unicellular hair ( $t^1$ ) arising from swelling of epidermis;  $t^2$ , capitate hair; *sto*, stoma;  $\times 160$ .

over the veins. As on the stem, there are two forms of hairs, unicellular and capitate. The unicellular hairs ( $t^1$ ) are stiff, very thick-walled and warty, varying in length up to 2 mm. and in diameter up to  $30\ \mu$ . The warts are rather more prominent than on the corresponding hairs of alfalfa. They arise from a conical rosette of elongated cells over a large intercellular space, resembling in outward appearance an emergence. The capitate hairs ( $t^2$ ), like those on alfalfa, consist of a stalk formed of a few cells in a single row and a club-shaped multicellular head.

*CALYX*.—The *outer epidermis* consists of simple cells with occasional wavy walls and numerous hairs both unicellular and capitate,



similar to those on the leaves and stem. The bristles, with papillae the whole length, end with a tuft of stiff unicellular hairs.

*Mesophyll*.—A single layer of crystal-bearing cells is conspicuous.

The *inner epidermis* is made up of wavy-walled cells and occasional hairs.

COROLLA.—The *epidermal cells* have very thin walls with papillae and striated cuticle toward the tip.

PERICARP.—The *epicarp* consists of sinuous-walled cells with scattered stomata. On the stem end the cell walls are thin, changing abruptly toward the tip to greatly thickened, sclerenchymatized and pitted walls.

The *mesocarp cells* are inconspicuous, with the exception of occasional scattered crystal-bearing cells.

SPERMODERM.—The *palisade cells* average  $45\ \mu$  in height (running up to  $55\ \mu$  over the radicle) and  $7\ \mu$  in breadth. A narrow light line lies about  $7\ \mu$  below the thin cuticle. They differ from the corresponding cells of alfalfa in that they are higher and the outer ends are flattened.

The *subepidermal cells* vary in height, but average  $10\ \mu$ . They are upward of  $20\ \mu$  broad and constricted in the center with lower ends broader than outer.

The *parenchyma* consists of thin-walled collapsed cells.

The ENDOSPERM and EMBRYO are of simple structure of no diagnostic importance.

### Alsike clover

Alsike clover (*Trifolium hybridum* L.), although indigenous to Europe, has become very common in America. The plant branches, with erect stems bearing 3-foliate toothed leaves on long petioles and pedicellate flowers forming a loose round head on a long peduncle. Like alfalfa, the plant is described as smooth, though hairs are evident under a lens and are of diagnostic importance with higher magnifications. The membranous 5-cleft calyx is much shorter than the delicate rose-pink tubular corolla, which after flowering becomes brown and withering-persistent. The pod differs from that of alfalfa in that it is straight and from red clover in that it is 2-4-seeded. The greenish brown seeds are smaller

than those of alfalfa and red clover, reaching a length of 1.5 mm., but in shape resemble closely those of red clover. They are flattened rounded triangular with one concave side.

### HISTOLOGY

**STEM.**—The *epidermal cells* are thin-walled, pitted, and longitudinally elongated, with numerous stomata. The cuticle shows longitudinal striations. Occasional hairs both unicellular and capitate are present, the warts usually being indistinct.

**Bast.**—Conspicuous crystal cells are found in the bast just below the chlorophyll-bearing cells.

**LEAF.**—The *upper epidermis* consists of isodiametric cells, averaging 30  $\mu$  in diameter, with straight thin walls.

In the *mesophyll* the cells accompanying the bundles contain crystals averaging 15  $\mu$  in length.

**Lower epidermis** (fig. 8).—The cells are similar to those of the upper surface, the walls toward the leaf margins becoming gently wavy. Occasional unicellular ( $t^1$ ) and capitate ( $t^2$ ) hairs are present, the former being indistinctly warty and arising from a slightly thickened epidermal cell. They vary in length up to 800  $\mu$ .

**CALYX.**—The *outer* and *inner epidermis*, with sinuous walls, bear capitate hairs similar to those on the leaf, also, at the base of the lobes and along their margins, unicellular thick-walled hairs with occasional indistinct warts.

**COROLLA.**—The *epidermis* consists of elongated sinuous-walled cells with striated cuticle, papillae being present at the tip of the petals.

**PERICARP.**—The *epicarp* consists of transversely elongated slightly sinuous thin-walled cells, scattered stomata, and, especially at the margins, capitate hairs.

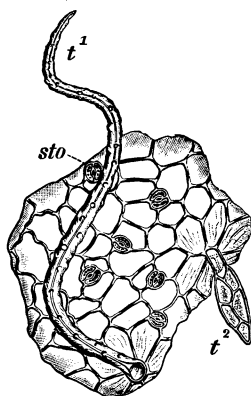


FIG. 8.—Alsike clover (*Trifolium hybridum*): lower epidermis of leaf with unicellular hair ( $t^1$ ), capitate hair ( $t^2$ ), and stoma (*sto*);  $\times 160$ .

The *mesocarp* is but a few cells thick, except at the margins. Scattered crystal-bearing cells occur either singly or in groups.

The *endocarp* is made up of a single layer of thin-walled elongated cells.

SPERMODERM.—The *palisade cells* are 30–50  $\mu$  in height and 7  $\mu$  in diameter, with a narrow light line about 7  $\mu$  from the outer end. They differ from the palisade cells of alfalfa in that they are slightly higher, and from those of red clover in that they are rounded (not flattened) on the outer ends.

The *subepidermal cells* are not distinguishable from those of alfalfa and red clover.

The *parenchyma* consists of thin-walled collapsed cells.

The ENDOSPERM and EMBRYO are of simple structure of no diagnostic importance.

### Identification in ground material

In a coarsely ground product, fragments of the leaves, flowers, pods, and seeds may be picked out and identified, but when powdered the unicellular hairs and crystals are the most conspicuous

	Alfalfa	Red clover	Alsike clover
Lower epidermis of leaf. ....	Wavy walls	Deeply sinuous walls with projections at angles and about stomata	Straight walls
Unicellular hairs. ....	Average diameter 15 $\mu$ , warts prominent	Average diameter 30 $\mu$ , warts prominent, arising from epidermal swelling	Average diameter 13 $\mu$ , warts indistinct

elements. Red clover may be distinguished from alfalfa and alsike clover by its larger, stiffer, and more numerous unicellular hairs arising from a swelling of the epidermis; alsike clover, from alfalfa and red clover, by the less distinct warts on the unicellular hairs.

The cell walls of the lower epidermis of the leaf are also characteristic, those of alsike clover being straight, of alfalfa simply

wavy, and of red clover very sinuous with projections at the angles and about the stomata.

A scheme for the identification of these three legumes by means of the epidermal cells of the leaf and the unicellular hairs is given in tabular form on the preceding page.

The palisade cells of the seed in alfalfa are not over  $35\mu$  high, whereas in alsike and red clover they average somewhat higher. In red clover the outer ends of these cells are flattened, but in alfalfa and in alsike clover they are rounded.

CHICAGO, ILL.